

Applicants: Dean Engelhardt et al.  
Serial No.: 08/486,066  
Filed: June 7, 1995  
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not apparent and thus involves undue experimentation.

Applicants disagree with the Examiner's position. The Examiner indicates that if a Sig moiety were attached to the 3' or 5' hydroxyl of a particular SM moiety, it would be a PM type attachment and not an SM attachment. This is not true when the labelling is at a terminal nucleotide. In such cases the labeling may be done at the 3' or 5' hydroxyl without involvement of the phosphate group. Thus, the chemistry noted by the Examiner involving formation of dialdehydes can be used for the attachment of Sig at the 3' and 5' positions as well as at the 2' position.

Moreover, the literature at the time of the invention disclosed the transformation of sugar moieties in nucleosides and nucleotides by various techniques and at various positions. By combining the prior art techniques with the teachings of the present application, one skilled in the art could certainly practice the invention at other positions of the sugar moiety other than the 2' position. For instance, it was known that branched sugar nucleosides could be prepared by the deaminative ring-contraction -- that is, 3'-amino-3'-deoxy -glucopyranosyl derivatives of uracil and adenine could be used for the synthesis of 3'-hydroxymethyl derivatives of 3'-deoxy or 2', 3'-dideoxy uridine and adenosine.

It has also been shown that it is possible to label RNA at the 3'-terminus with fluorochrome. Thiosemicarbazides derived from tetramethylrodamine isothiocyanate (TRITC) and fluorescein isothiocyanate (FITC) were coupled to the aldehydes generated by periodate oxidation of RNAs. This technique results in breakage of the bond between the 2' and 3' positions and insertion of the label. Similarly, ferritin has been covalently attached to the sugar moiety of RNA via an avidine-biotin conjugate. The 2' and 3' hydroxyls of RNA are oxidized by periodate to dialdehydes and then coupled to one of the amino groups of 1,5-diaminopentane by Schiff base formation and subsequent  $\text{NaBH}_4$  reduction. NHS-biotin is attached to the resulting RNA to form RNA-NHS-biotin conjugates. Again the labelling is at a position other than the 2' position.

Applicants also disagree with the Examiner's statement that the only disclosed chemistry for attachment of Sig to the sugar moiety is on page 53, first full paragraph. Contrary to the Examiner's statement, Example V on page 57 of the application sets forth an exemplary labeling of the phosphate moiety of oligonucleotides with biotin and polybiotinylated poly-L-lysine in the presence of carbodiimides; this chemistry could also be used for the labelling of the sugar moiety with Sig. The example references an article by Halloran and Parker, *J. Immunol.*, 96: 373 (1966), for the specifics in covalently conjugating

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a protein with a polynucleotide at the PO<sub>4</sub> groups. As noted in the article, the OH group of the sugar residue could also be reacted with the protein carboxyl groups in the presence of carbodiimides. Accordingly, the application provides other chemistry for the labelling of the sugar moiety other than that on page 53.

The Examiner further maintains that the scope of claim 238 is broader than the available SM labeling methods because the point of attachment of Sig to SM is not limited and the attachment is not limited to a covalent character. Applicants respectfully disagree with the Examiner. As discussed above, techniques were available in the art which could be used for the attachment of groups to various positions of SM. With respect to the covalent nature of the attachments, applicants direct the Examiner to the language of claim 238 wherein it is stated that Sig is covalently attached to SM directly or through a linkage group.

Furthermore, applicants wish to point out that the disclosure of the present application has already been deemed by the U.S. Patent Office as adequate support for claims wherein Sig is attached to the sugar moiety of polynucleotides. United States Patent No. 5,260,433, which issued November 9, 1993, derived from the same parent application as that of the present application and hence has the same disclosure. In this patent, claims were allowed wherein Sig is attached to the sugar moiety: see for example, claim 1.

In sum, applicants respectfully disagrees with the Examiner's position and contend that in light of the level of knowledge in the art and the teachings set forth in the present application, a person of ordinary skill would possess the requisite knowledge to attach the Sig moiety to SM at various positions with a reasonable expectation of success.

The Examiner also rejected claims 238-297 and 299-307 under the judicially created doctrine of obviousness-type double patenting as unpatentable over claims 1-24 of U.S. Patent No. 5,260,433. Applicants are in the process of obtaining a Terminal Disclaimer from the assignee to alleviate this rejection.

In light of the above, applicants respectfully request withdrawal of the various rejections set forth in the June 25, 1996 Office Action and the issuance of a Notice of Allowance for the application.

Lastly, the Examiner requested copies of the PTO forms 1449 that were submitted with applicants' September 11 and October 25, 1995 Information Disclosure Statements (IDS). The requested forms from applicants' September 11, 1995 IDS are attached hereto as


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Exhibit A. Applicants are in the process of obtaining copies of the forms from their October 25, 1995 IDS and will forward them to the Examiner in the near future.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney requests that he be contacted at the number provided below. (Please note that the address and telephone number for applicants' attorney has changed; a Change of Address communication is being concurrently submitted with the present response.)

In addition to the \$930 fee for the extension of time, \$ 682 is due for the addition of new claims. Authorization is hereby given to charge these fees to Deposit Account No. 05-1135. If any other fee is deemed necessary, authorization is hereby given to charge the amount of such fee to Deposit Account No. 05-1135.

Respectfully,

  
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